THE ALIMENTARY CANAL OF *PHYLLOPHAGA GRACILIS* BURM.*†

FRED WALKER FLETCHER.

INTRODUCTION.

The following study is a direct result of interest developed in a course of entomology given by Dr. C. H. Kennedy, on "Morphology and Development of Insects."

The material used in this work was collected in anticipation of such a study while the author was employed temporarily at the Asiatic Beetle Laboratory, at Westbury, Long Island, New York. The choice of *Phyllophaga gracilis*, Burm. for this problem was due to availability of specimens taken at a trap-light. This particular species is about one-half inch in length, elongate oblong in shape and pale brown in color. In the summer these beetles are found in Eastern Canada, Eastern and Southern United States, even extending as far west as Texas (5)‡. A considerable number of specimens was fixed in Kahle's solution after which they were transferred to and preserved in 70% alcohol.

The author acknowledges the helpful suggestions and criticisms of Dr. C. H. Kennedy under whose direction this study was made.

GROSS ANATOMY OF THE DIGESTIVE SYSTEM.

GENERAL DISCUSSION.

The alimentary canal is one and one-half times longer than the body, in correlation with the phytophagus habits of this beetle. Morphologically the alimentary canal is divisible into three primary regions according to their embryonic origin. The fore-intestine (Stomodaeum) arises as an anterior ectodermal invagination; the hind-intestine (Proctodaeum) arises as a similar posterior invagination; the mid-intestine (Mesenteron or Ventriculus) which connects the two, develops as an endodermal sac from a proliferation of rings of endodermal cells, one around the posterior end of the fore-intestine and the other around

* Contribution No. 99 from the Department of Zoology and Entomology, Ohio State University.

†A thesis presented as a partial requirement for the Degree of Master of Arts.

‡Numbers in parentheses refer to literature cited in bibliography at end of paper.

the anterior end of the hind-intestine (2). These primary regions with their differentiations may be tabulated as follows: (Fig. 1, Plate I).

Fore-intestine.
1. Pharynx.
2. Oesophagus.
3. Crop.
4. Oesophageal valve.

Mid-intestine.
1. Stomach.

Hind-intestine.
1. Pyloric valve.
2. Malpighian tubules.
3. Proximal ileun.
4. Distal ileun.
5. Colon.
6. Rectum.

GROSS STRUCTURES OF FORE-INTESTINE.

The fore-intestine is a rather short, slender tube which comprises a little over one-eighth of the total length of the entire canal. It extends from the mouth back to the beginning of the mesothorax.

The *pharynx*, the initial region of the alimentary canal just posterior to the mouth, is evident as a slight dilation of the tract in the head.

The *oesophagus* is a short narrow tube connecting the pharynx to the crop, lying in the most anterior part of the prothorax. The only essential difference between this region and the two it connects is size, the oesophagus being smallest. Like the pharynx, it has apparently no function other than conduction.

The *crop* is present as a dilation of the hinder portion of the oesophagus, but not being large and conspicuous in this insect, it is doubtful whether it functions as a food reservoir, as does a true crop. This portion of the canal lies entirely in the posterior part of the prothorax.

The *oesophagal valve* marks the separation between the fore- and mid-intestine by a constriction. This constriction may be found at the divison between the pro and mesothorax; functionally this valve serves to prevent regurgitation.

GROSS STRUCTURE OF MID-INTESTINE.

The stomach or mid-intestine comprises a little more than half the total length of the alimentary canal in this species of insect. It extends as a straight tube, nearly uniform in diameter, to the beginning of the abdomen where it curves a little to the left and then continues to the fourth abdominal segment. Here it curves abruptly to the right and extends back to the second abdominal segment. At this point it turns caudad and in the third abdominal segment makes a complete loop which lies underneath the preceding portion and terminates very abruptly at the pyloric valve.

Fundamentally the ventriculus has the power of secretion and of absorption.

GROSS STRUCTURES OF THE HIND-INTESTINE.

The hind-intestine occupies a little more than a fourth of the total length of the tract and is clearly differentiated into its various parts.

The *pyloric-valve*, located at the union of the mid-intestine with the hind-intestine, serves to close the posterior end of the stomach. It is recognizable as a slight constriction just antecedent to the Malpighian tubules.

The *Malpighian tubules* are attached to the canal just posterior to the pyloric valve and are four in number. Two of these tubules arise from a common opening, while the other two arise separately.

The *proximal ileum* is comparatively short and appears much the same as the mid-intestine. This portion of the canal is posterior to the Malpighian tubules and lies in the fourth abdominal segment.

The *distal ileum* is much the larger of the two parts of the ileum. In general outline it resembles a mammalian stomach and is the most conspicuous region of the hind-intestine. Its anterior margin is well defined as it arises abruptly from a constriction in the proximal ileum. Its caudal margin is likewise definitely narrowed where it joins the colon. The distal ileum normally lies in the body with the anterior end more caudad than the posterior one. As this region of the tract leaves the proximal ileum, it turns abruptly to the left and extends forward with the posterior end resting on the coiled part of the ventriculus. The wall of the distal ileum is quite transparent and internal papillate processes are visible from the exterior. Most of this part of the canal lies in the third and fourth abdominal segments. Its function is obscure.

The *colon* is a small tube linking the distal ileum with the rectum. As it arises from the distal ileum it takes a rather irregular path back to the rectum, with which it merges gradually. The colon is uniform in diameter throughout. Its function is mainly conduction.

The *rectum* is just an enlargement of the colon and lies in the fifth and sixth segments of the abdomen. Both circular and longitudinal muscles are evident on its surface. Like the colon its function is conduction.

Histological Structure of the Alimentary Canal.

Histological structure of the fore-intestine.

Histologically the fore-intestine reveals a similarity of make-up throughout its various parts. On examination the following tissues from within outwards can be demonstrated in its wall: (1) Intima of cuticula of chitin; (2) Epithelium of hypodermal cells; (3) Longitudinal muscles; (4) Circular muscles, and (5) "Peritoneal membrane" of connective tissue cells.

The innermost layer is the cuticula of chitin which is homologous with the cuticula of the body wall and is secreted by the hypodermal epithelium. This intima forms a rather thin, non-cellular, almost transparent layer which lines the entire fore-intestine (Figs. 2, 4 and 5, Plate I). In the region of the pharynx, however, it is thicker than in the other parts, being covered with small chitinous spines which project more or less caudad (Fig. 2, Plate I).

The epithelium of hypodermal cells which form the layer of tissue just outside the intima is composed of flattened irregular cells. Their cell-walls are usually quite evident although a basement membrane was not visible.

Immediately outside the epithelium may be found isolated bundles of longitudinal muscle tissue. A cross-section of the fore-intestine

demonstrates three such groups of muscles situated at the bases of folds in the epithelium.

Surrounding the longitudinal muscle layer is a more conspicuous layer of circular muscles, especially so around the anterior part of the pharynx and around the oesophagus. Elsewhere along the fore-intestine these muscles appear as scattered strands.

The outermost part of the fore-intestine has traces of connective tissue known as "peritoneal membrane." This consists of apparently structureless tissue in which an occasional nucleus makes its appearance.

At the point of junction of the fore- and hind-intestine, there is an *oesophageal valve* (Fig. 4, Plate I). This structure is formed by the wall of the fore-intestine being prolonged into the lumen of the mid-intestine. This projection of the fore-intestine then becomes reflected upon itself and passing forward unites with the stomach wall, (2). At this point the intima of the fore-intestine disappears. The epithelial cells of the inner part of this fold are much the same as those of the crop, being flattened and more or less irregular in shape.

The epithelial cells of the reflected area in the valve are much larger than those just mentioned and assume the shape of columnar epithelium.

The depression formed by the extension of the fore-intestine into the mid-intestine is filled with circular muscles. These are very numerous and serve to close the valve.

Just outside these muscles are ten or twelve strands of longitudinal muscles which extend from the epithelium of the fore-intestine across to the epithelium of the mid-intestine. Scattered between both ends of these muscles may be found numerous circular muscles. At this point the longitudinal and circular muscles reverse their positions in respect to each other, i.e. at the point where the mid-intestine originates, the longitudinal muscles shift to the outside of the circular muscles. The reverse is true in the fore-intestine. (See Fig. 4.)

The "peritoneal membrane" is present in this region.

HISTOLOGICAL STRUCTURES OF THE MID-INTESTINE.

The mid-intestine in *Phyllophaga gracilis* is an elongate tube which is marked anteriorly by the oesophageal valve, and posteriorly by the pyloric valve. This section of the canal is quite uniform in shape throughout, except that the posterior half is thrown into folds.

The structure of the mid-intestine is markedly different from that of the fore-intestine. In the ventriculus there is no intima, the relative positions of the circular and longitudinal muscles being reversed.

Histological examination of the stomach gives the following sequence of tissues: (1) epithelium of endoderm cells supported by a basement membrane. (2) circular muscles. (3) longitudinal muscles, and (4) "peritoneal membrane" of connective tissue. (Figs. 6 and 7, Plate I).

The cells of the entire epithelium vary greatly at times in size and shape; they may be almost cuboidal after a period of secretion; or they may be decidedly columnar during the resting stage, depending upon their physiological condition.

Secretion seems to be of the holocrine type, where the entire contents of the cell bursts out into the lumen of the intestine, after which the cell is replaced by new cells. In this particular insect these cells seem to be replaced either from nidi, (nests of embryonic tissue that lie below the epithelium) or from small replacement cells that rest on the basement membrane at the base of the epithelium. These cell nests are rather peculiar in that they lie completely below the epithelium, their definite relationship to digestion being as yet obscure. Further study may possibly reveal a condition similar to that found by Lewis in *Passalus cornutus*. The replacement cells already referred to are recognizable as small triangular shaped cells at the base of the epithelial layer. They present a varying degree of sizes, and in some sections, their function of replacement can hardly be doubted. It is hoped that further study will throw some light on these problems.

Surrounding these nidi and just outside the basement membrane may be found the circular muscle layer. This layer usually forms a rather conspicuous and continuous ring of tissue around the canal as viewed from a cross-section. (Fig. 6, Plate I). These muscle fibers are more or less interlaced at their ends.

Immediately outside the circular muscle layer are isolated strands of longitudinal muscles that are not nearly so abundant as the circular muscles.

A rather conspicuous amount of connective tissue is present in both the cross and longitudinal sections. (Fig. 7, Plate I).

HISTOLOGICAL STRUCTURES OF HIND-INTESTINE.

The hind-intestine which comprises a little more than one-fourth the total length of the alimentary tract, is differentiated considerably in its several parts. Anteriorly it is marked by the pyloric valve and posteriorly by the anus. Histologically this division of the canal presents from within outwards the following tissues: (1) intima; (2) Hypodermal epithelium resting on a basement membrane; (3) Circular muscles; (4) Longitudinal muscles, and (5) Connective tissue.

The *pyloric valve* represents the anterior limit of the hind-intestine. (Fig. 8, Plate II). The mid-intestine terminates abruptly and along the posterior margin of the columnar epithelium there is a row of small cuboidal cells. Immediately below this point of the tract there arises a ring of greatly elongated cells which extend down into the hind-intestine. This group of hypodermal cells is known as the pyloric valve.

The inner margin of the valve is lined with intima and makes its origin at the point of division between the mid- and hind-intestine.

The epithelium of the valve is bounded on the outside by well-developed circular muscles which function in closing the valve.

The basement membrane is indistinct.

The *Malpighian tubules* arise as invaginations of the hind-intestine just posterior to the pyloric valve. (Fig. 8, Plate II). The epithelial cells at origin of the tubules are rather narrow and elongate but they soon assume their characteristic cuboidal shape. The nuclei throughout the tubules are quite large ovate structures. Cross sections of the tubules show a decided difference in their sizes; number of epithelial

cells in such sections varying from five to fourteen or fifteen. (Figs. 9, 10 and 11, Plate II).

At the mouth of these tubules and extending into them for a short distance is a very delicate layer of intima. Elsewhere in them they are lined with a striated border. In some sections however this striated border is indistinct.

On the outside the Malpighian tubules are bounded by a narrow layer of connective tissue.

The *ileum* is the intial portion of the hind-intestine. It is bounded on the anterior side by the pyloric valve and on the posterior margin by the colon. In gross structure and histological make-up the ileum is divided into a proximal and distal region.

The *proximal division* of the ileum is shorter and narrower than the distal ileum and very thin walled. (Fig. 3, Plate I). The anterior margin gives rise to the Malpighian tubules which the posterior one is constricted where it joins the distal part of the ileum.

Internally the proximal ileum is lined with a very delicate layer of intima. Outside this layer of chitin is a layer of epithelium composed of very irregular cells which possess rather large ovoid nuclei. This layer of cells is thrown into numerous folds at each extremity of this portion of the ileum. The basement membrane is not visible.

The circular muscle layer is quite prominent and usually composed of two or three thin strands of muscle tissue.

Along the outside of this layer are scattered numerous isolated strands of longitudinal muscle tissue.

"Peritoneum" is also evident in some sections.

The *distal ileum* of *Phyllophaga gracilis* is without a doubt the most outstanding structure in the whole alimentary canal. It is unusual, both in its gross and histological anatomy. In size it is much larger than the proximal ileum, being over four times as long and twice as wide. The anterior margin of the distal ileum arises from a constriction at the posterior end of the proximal ileum. It at once expands to its normal size, having a diameter greater than that of any other part of the canal. Its caudal end narrows quite abruptly as it joins the colon but no indication of a valve is present at either end.

On examining this region microscopically, a very thin, delicate, almost transparent intima is found lining it throughout. The epithelium just outside possess cuboidal cells, except in some of the fold-like projections where they are more or less flattened and irregular in form.

The epithelium of the distal ileum is traversed by six longitudinal rows of papillate processes which extend into its lumen (Fig. 12, Plate II). The number of these projections per row vary according to their location on the ileal wall. Along the concave wall of this structure the protrusions vary in number from eight to ten, while on the opposite convex side, the variation is between twelve and sixteen. The total number of papilliform processes for the entire organ may vary from sixty to almost a hundred. These processes also vary considerably in size, those found in the middle portion of the distal ileum being much larger than those at each of its ends.

The intima enveloping these protrusions is covered with small spines which seem to aid in holding the cone-like mass of non-cellular

material which fits down over each one. (Fig. 13, Plate II). This matrix assumes a deep blue color when stained with Delafield's Haemotoxylin. In it appear clear ovate objects which may be protozoa. A single median longitudinal section, seven micra in thickness, may contain nearly a hundred of these objects. It is possible that bacteria may produce this anomalous medium and that there might be a symbiotic relationship existing between protozoa and bacteria in this connection.

The circular layer of muscles surrounding the hypodermal epithelium of the distal ileum is quite well developed. Groups of them may be seen at the bases of small folds in the hypodermis in longitudinal sections. They are however found elsewhere as scattered strands beneath the epithelium.

The longitudinal muscles on the other hand are not as well developed and only an occasional strand is found.

Traces of "peritoneal membrane" are also present.

The *colon* is defined anteriorly by the distal ileum and posteriorly by the rectum, with which it gradually merges. The colon is a long slender tube with a diameter of about one-third that of the distal ileum.

The intima of this division of the tract is somewhat thicker than that in the ileum.

The epithelial cells of the colon are cuboidal in shape, possessing very large nuclei. A basement membrane is evident.

That side of the colon comparable to the concave side of the distal ileum is a short row of a dozen or so large spines which project caudad into the lumen of the colon. Also there are six large longitudinal folds in the epithelium of this region which are quite evident in cross-sections. (Fig. 14, Plate II).

The circular muscle layer of the colon is well developed and cross-sections of this part of the canal show a continuous band of them surrounding the epithelium.

The longitudinal muscles, however, are very poorly developed, only from two to four isolated groups being evident in a cross-section.

The *rectum* is very similar to the colon in make-up. The transition from one to the other takes place gradually. Externally the rectum is a dilation of the posterior end of the alimentary canal.

The intima and epithelium, as in the colon, are thrown into six large longitudinal folds and are known as rectal pads. The intima, however, is very thick, and in this respect, differs decidedly from the colon. The epithelial cells are of the cuboidal type and rest on a basement membrane that shows up more definitely here than in any other part of the canal.

The circular muscles are well-developed in this section of the alimentary tract, five of six layers of them being present.

The longitudinal muscles as in the rectum are few and inconspicuous. A small number of isolated groups comparable to those in the colon are present in cross-sections of this region. (Fig. 15, Plate II).

The "peritoneal membrane" of connective tissue appears much the same as it does in other divisions of the tract.

CONCLUSION.

The alimentary canal of *Phyllophaga gracilis* is of medium length, corresponding to its phytophagus habits. In correlation with other insects this canal is made up of three primary regions. The fore-intestine (Stomodaeum) and the hind-intestine (Proctodaeum) both ectodermal in origin and the mid-intestine (Mesenteron) which has an endodermal origin.

These three regions, however, are more or less specialized and differentiated, exhibiting the following modifications:

Fore-intestine:—Pharynx, oesophagus, crop and oesophageal valve.

Mid-intestine:—Entirely stomach.

Hind-intestine:—Pyloric valve, Malpighian tubules, proximal ileum, distal ileum, colon and rectum.

This study of the alimentary canal needs considerably more attention before a thorough understanding can be secured. The physiology of the digestive system should be worked out, in order to learn the exact relationships of the nidi and replacement cells, in this connection. The nidi afford considerable interest since they have in many cases broken not only through the basement membrane but also through the surrounding muscle layers. Such a study would be the best possible way to determine the type of secretion and absorption.

The condition existing in the distal ileum likewise requires an even more detailed study with fresh material, before a possible solution of its nature can be given. In this part of the ileum the papillate processes surrounded by an anomalous medium, filled with clear ovate objects resembling protozoa of the Microsporidian type present the most interesting situation in the whole alimentary canal of this beetle. It is hoped that further study will give a clue to the identity of these structures.

BIBLIOGRAPHY.

1. **Comstock, J. H.** 1924. An Introduction to Entomology.
2. **Imms, H. D.** 1924. A General Textbook of Entomology.
3. **Lewis, H. C.** 1926. The Alimentary Canal of Passalus. Ohio Jr. Sci., Vol. XXVI, No. 1, pp. 11-24.
4. **Packard, A. S.** 1903. A Textbook of Entomology.
5. **Sim, A. J.** 1928. Phyllophaga (Scarabaeidae) of the United States and Canada. Cir. No. 145, State Dept. of Agric. N. J.
6. **Woods, W. C.** 1918. The Alimentary Canal of the Altica bimarginata Say (Coleoptera). Ann. Ent. Soc. Amer., Vol. XI, No. 3, pp. 283-318.

EXPLANATION OF PLATES.

PLATE I.

Fig. 1. A dorsal view of the Alimentary Canal. Col.—colon; crop; D. Il.—Distal Ileum; Mal. T.—Malpighian Tubules; M. Int.—Mid-intestine; Oes.—Oesophagus; Oes. V.—Oesophageal valve; Ph.—Pharynx; P. Il.—Proximal Ileum; Py. V.—Pyloric valve; Rec.—Rectum.

Fig. 2. Cross-section through pharynx. C. Mus.—Circular Muscle; Epi.—Epithelium; Int.—Intima; L. Mus.—Longitudinal muscle; Sp.—Spine.

Fig. 3. Cross-section of Proximal ileum. Oil emersion drawing. C. Mus.—Circular muscle; Epi.—Epithelium; Int.—Intima; L. Mus.—Longitudinal Muscle.

Fig. 4. Longitudinal section through the oesophageal valve. C. Mus.—Circular Muscle; Epi.—Epithelium; Int.—Intima; L. Mus.—Longitudinal Muscle; Oes. V.—Oesophageal valve; P. mem.—"Peritoneal membrane."

Fig. 5. Cross-section through oesophagus. C. mus.—Circular muscle; Epi.—Epithelium; Int.—Intima; L. mus.—Longitudinal muscle.

Fig. 6. Cross-section of mid-intestine. C. mus.—Circular muscle; Epi.—Epithelium; F. T.—Fat tissue; L. mus.—Longitudinal muscle; Nidus; R. cell—Replacement cell.

Fig. 7. Longitudinal section through mid-intestine. C. mus.—Circular muscle; Epi.—Epithelium; F. T.—Fat tissue; L. mus.—Longitudinal muscle; Nidus.

PLATE II.

Fig. 8. A longitudinal composite section through region of pyloric valve. C. mus.—Circular muscle; Epi.—Epithelium; Int.—Intima; Malp. Tub.—Malpighian Tubule; L. mus.—Longitudinal muscle; Py. valve—Pyloric valve.

Fig. 9. Cross-section through Malpighian tubule near extremity. Oil emersion drawing.

Fig. 10. A typical cross-section through a Malpighian tubule. Oil emersion drawing.

Fig. 11. Cross-section of Malpighian tubule near its origin. Oil emersion drawing. Epi.—Epithelium; P. mem.—"Peritoneal membrane" or connective tissue; St. B.—Striated border.

Fig. 12. Cross-section through distal ileum. Pap. Proc.—Papillate Process.

Fig. 13. Longitudinal section through distal ileum showing a papillate process. Oil emersion drawing. A. Med.—Anomalous medium; C. mus.—Circular muscle; Epi.—Epithelium; Int.—Intima; L. mus.—Longitudinal muscle; M. S.—Probably protozoa of the Microsporidian type; Sp.—Spine.

Fig. 14. Cross-section through the colon. C. mus.—Circular muscle; Epi.—Epithelium; Int.—Intima; L. mus.—Longitudinal muscle.

Fig. 15. Cross-section through the rectum. C. mus.—Circular muscle; Epi.—Epithelium; Int.—Intima; L. mus.—Longitudinal muscle.

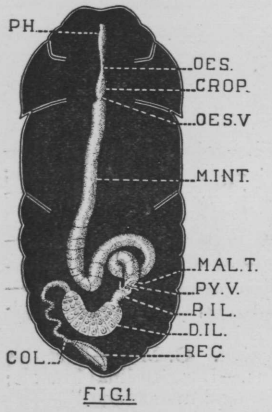

FIG.1.

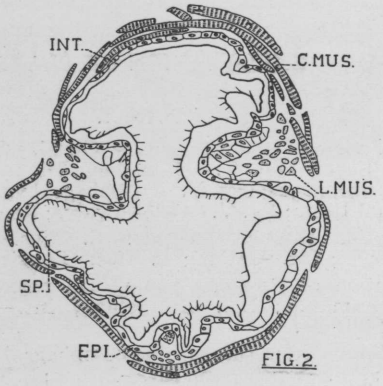

FIG.2.

FIG.3.

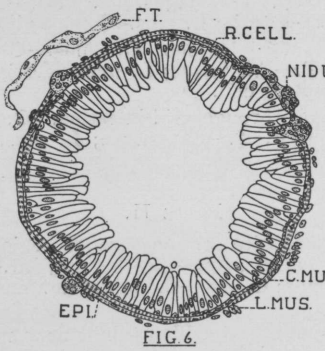

FIG.6.

FIG.5.

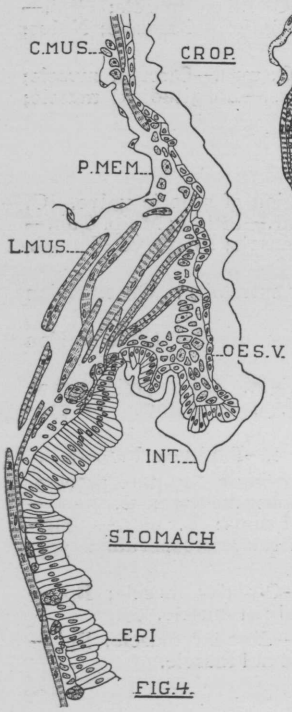

FIG.4.

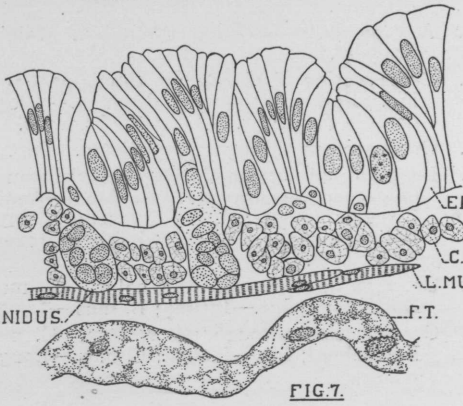

FIG.7.

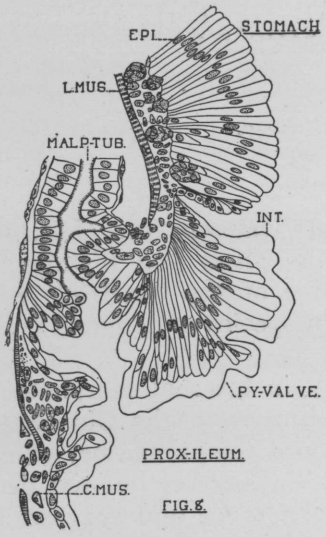

FIG. 8.


FIG. 9.

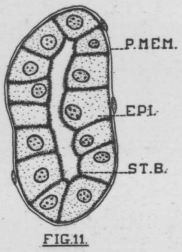

FIG. 11.


FIG. 10.

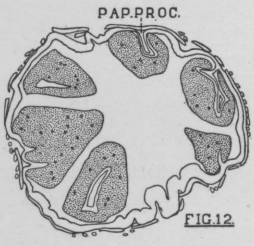

FIG. 12.

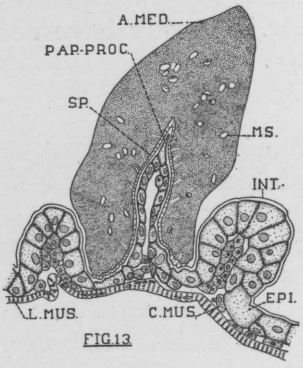

FIG. 13.

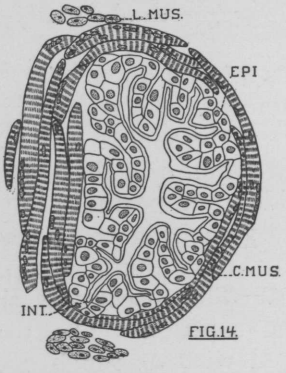

FIG. 14.

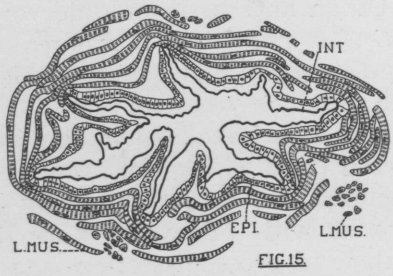

FIG. 15.